# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Cyclodextrin glucanotransferase (EC 2.4.1.19) is an industrially important enzyme that produces α-, β- and γ-cyclodextrins (CDs) from starch through an intramoleculer transglycosylation reaction. The α-, β- and γ-CD have closed ring structures with six, seven and eight glucose units joined by α- 1,4-glucosidic bonds respectively. The exteriors of CDs are hydrophilic while the interiors are hydrophobic, enabling it to easily form inclusion complexes with either organic or inorganic molecules. The encapsulated guest undergoes advantageous physical and chemical changes, such as improved stability and water solubility in a variety of fine organic and inorganic compounds, sharply reducing volatility, chemical, thermal and light reactivity of guest molecules, stabilization and protection of sensitive hosts such as flavors, odor and aroma. As a result, CDs and its derivatives have wide application in today's industries such as food, cosmetics, chemicals, environmental, agricultural, cosmetics, pharmaceuticals and toiletries.

The CGTase enzyme is generally found in bacteria and a wide variety of bacteria have been determined as CGTase producers, namely aerobic mesophilic bacteria, aerobic thermophilic, anaerobic thermophilic and aerobic alkalophilic bacteria. Various genera of bacteria that are known as CGTase producer includes *Bacillus*, *Klebsiella*, *Pseudomonas*, *Brevibacterium*, *Thermoanaerobacterium*, *Corynebacterium*, *Micrococcus*, and *Clostridium* etc (Gawande *et al.,* 1999).

Most CGTases produce a mixture of α-, β- and γ-CD in different ratios, depending on the origin of the CGTase as well as the reaction conditions. CGTase is classified into three different types, α-CGTase, β-CGTase and γ-CGTase according to the major CD produced. As a result, industrial production process of CDs and the subsequent separation process are rather elaborate and costly as expensive purification procedures are needed. Furthermore, there is the consideration of solvent toxicity, flammability and the need for a solvent recovery process which is an added disadvantage. Besides, the complete removal of solvent from the CDs is expensive, limiting the use of CDs in the pharmaceutical and food industries (Biwer *et al.,* 2002).

Therefore, the availability of CGTase enzymes that are capable of producing an increased ratio of one particular type of CD and also with reduced product inhibition would help to avoid using expensive and environmentally harmful procedures involving organic solvents. On top of that, it is also desirable to develop a novel CGTase that is better to produce CD in high proportion from starch for industrial and biochemical studies. In order to achieve this, genetic engineering or molecular biotechnology technique will facilitate the process of obtaining better enzymes.

The attractive feature of CGTase from *Bacillus* sp. G1 is that it predominantly produced β-CD (89%) from tapioca starch and this high yield can be increased to 100% yield of β-CD with the addition of 4% (v/v) Triton X-100 (Ho *et al*., 2005). Therefore, CGTase from *Bacillus* sp. G1 can be considered as a good model enzyme for further studies of β-CD production. It is also a potential candidate for commercialization and industrial production of β-CD due to its stability, high specificity in β-CD production, versatility and ease of handling.

## 1.2 Objectives of the Research

The main objective of this research is to clone a cyclodextrin glucanotransferase gene (CGTase) from *Bacillus* sp. G1 and to express it.

## 1.3 Scope of Research

There are five scopes in this research:

(a) Bacterial 16S rRNA Identification
(b) Isolation and cloning of CGTase gene from *Bacillus* sp. G1
(c) Analysis of nucleotide sequence of CGTase gene and its deduced amino acid sequence
(d) Expression of CGTase in *E.coli*
(e) Characterization of the partially purified recombinant CGTase